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Comparative Neurobehavioral Study of a Polybrominated Biphenyl-Exposed Population in Michigan and a Nonexposed Group in Wisconsin

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An analysis of findings regarding the prevalence and time course of symptoms and the results of neurobehavioral testing among Michigan and Wisconsin dairy farmers, is reported. Reviewed are: (1) differences in the prevalence of neurological symptoms at the time of examination; (2) differences in the incidence and time course of symptoms for the period 1972–1976; (3) differences among populations and subgroups (sex and age) regarding performance test scores; (4) correlations between performance test scores and neurological symptoms; and (5) correlations between serum PBB levels as indicators of exposure and performance tests and neurological symptoms.

This is a report of findings regarding performance test scores and neurological symptoms in individuals suspected of being exposed to polybrominated biphenyls (PBB) in Michigan (primarily dairy farm residents) and within a control population of dairy farm residents in Wisconsin. Reviews of PBB toxicity and of the Michigan PBB incident have been published elsewhere (1–5). The present study was part of comprehensive medical field survey conducted by our Laboratory between November 1976 and March 1977 in Michigan and Wisconsin.

The major questions addressed in this report include: (1) the prevalence of neurological symptoms in the Michigan population exposed to PBB and its difference from that in the Wisconsin, non-PBB exposed group; (2) differences in the incidence and time course of symptoms in the Michigan and Wisconsin populations for the period 1972-1976; (3) differences in standard performance test scores; (4) correlation between prevalence of symptoms (if found) with the results of objective performance test aimed at assessing neuropsychological dysfunction; (5) contributions of sex, age, and education to the prevalence of neurological symptoms and/or the performance test scores; (6) correlations of performance test results with serum PBB levels; and (7) correlations between specific neurological symptoms and serum PBB levels.

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Materials and Methods

Subjects

The symptoms of 644 adults from Michigan and 153 from Wisconsin were analyzed for prevalence and incidence of neuropsychological symptomatology. The characteristics of these two populations and patterns of symptoms other than neurological are discussed elsewhere in this issue (6).

The subsample examined by means of performance tests were 102 males and 68 females from Michigan and 50 males and 43 females from Wisconsin. The subsample was chosen at random during comprehensive crosssectional clinical surveys in these two states. All examinees were whites. The educational level ranged from a few years of schooling to individuals with college degrees. The distribution of educational levels in the two populations is almost identical with a peak at a unit be-

tween 3–3.9, a rating corresponding to a high school degree or a little higher. The peaks of the age distribution for Michigan and Wisconsin were located at 35–44 and 45–54 years of age. The weight attributed to educational level and age in these tests has been discussed elsewhere (*7*). Other factors being equal, the average scores of the Wisconsin sample are expected to be lower than the Michigan sample because of these differences (not statistically significant) in age. In a few cases and for a variety of reasons (such an interruption of the testing for compelling reasons or physical handicap) data for individual tests in some are missing.

Neurobehavioral Tests

Block Design (BD), Digit Symbol (DS), and Embedded Figures (EF) tests were used in the assessment of performance of the populations studied. The Block Design test is widely used in the evaluation of the effects of neurotoxic substances. The test provides a quick and objective measure of the subject's ability to visualize spatial relations. The examinee is presented with a set of colored cubes. Each cube has sides which are red, white, and half red and half white. The task requires arranging the cubes so that the top surface reproduces a displayed design. In the first six trials, only four of the cubes are used and the designs to be reproduced are relatively simple. In the last four trials, nine cubes are used and the designs are more complex. The score is based on the number of correctly completed trials as well as the speed with which the test is accomplished.

The Digit Symbol test assesses the subject's ability to associate symbols according to a code. The subject is given a list in which symbols are associated with digits from 1 to 9 and is asked to enter the symbols into the blank spaces next to a list of random digits on a form. The score is the number of symbols correctly entered in 90 sec.

The Embedded Figures test has been extensively used in neurological research, particularly during World War II. The test, consisting of four sets of superimposed outlines of common objects, assesses visual perception in masked conditions. The score is the total number of objects successfully identified in the four sets.

Reliability and Validity of Tests

Test-retest reliability data for the Block Design test and for the Digit Symbol test have been provided by Wechsler (*8*). The test-retest correlation coefficient is between 0.82 and 0.85 for ages 18–54 and 0.80 for ages 16–24 respectively. The Embedded Figures test has been developed in our laboratory in studies of over 600 subjects classified by age, sex, race, education, occupation, geographic area, rural or urban living and native language (English and Spanish). This battery of tests has been shown to be sensitive in detecting subclinical central nervous system dysfunction as a result of excessive exposure to lead (*7*).

All test scores correlated positively and significantly among themselves within the control and test population. Age correlated significantly and negatively with all behavioral test scores in both groups. Matrices of test intercorrelations are given in Tables 1 and 2.

Table 1a. Matrices of intercorrelations between age, education, and performance test scores (males, three age groups, Wisconsin).

Age, yr	N		Correlations						
			Age	Educ.	BD	DS	EF	DDE	PPB
0–29	16	Age	+1.00	−0.08	+0.24	−0.09	+0.12		
		Educ.		+1.00	−0.23	+0.49	+0.68		
		BD			+1.00	−0.37	+0.20		
		DS				+1.00	+0.36		
		EF					+1.00		
		DDE						+1.00	
		PBB							+1.00
30–49	14	Age	+1.00	−0.39	−0.07	−0.39	−0.29		
		Educ.		+1.00	+0.24	+0.56	+0.17		
		BD			+1.00	+0.53	+0.41		
		DS				+1.00	+0.15		
		EF					+1.00		
		DDE						+1.00	
		PBB							+1.00
≥ 50	22	Age	+1.00	−0.19	−0.41	−0.49	−0.63		
		Educ.		+1.00	+0.28	+0.49	−0.12		
		BD			+1.00	+0.60	+0.57		
		DS				+1.00	+0.46		
		EF					+1.00		
		DDE						+1.00	
		PBB							+1.00

Table 1b. Matrices of intercorrelation between age, education, and performance test scores (females, three age groups, Wisconsin).

Age, yr	N		Correlations						
			Age	Educ.	BD	DS	EF	DDE	PBB
0–29	7	Age	+1.00	+0.62	−0.50	−0.58	−0.69		
		Educ.		+1.00	−0.71	−0.47	+0.37		
		BD			+1.00	+0.10	+0.22		
		DS				+1.00	+0.26		
		EF					+1.00		
		DDE						+1.00	
		PBB							+1.00
30–49	18	Age	+1.00	−0.12	−0.18	−0.45	−0.15		
		Educ.		+1.00	+0.08	−0.23	−0.08		
		BD			+1.00	+0.12	+0.31		
		DS				+1.00	+0.27		
		EF					+1.00		
		DDE						+1.00	
		PBB							+1.00
≥ 50	16	Age	+1.00	−0.15	−0.61	−0.44	−0.38		
		Educ.		+1.00	+0.28	+0.56	+0.48		
		BD			+1.00	+0.25	+0.63		
		DS				+1.00	+0.22		
		EF					+1.00		
		DDE						+1.00	
		PBB							+1.00

Table 2a. Matrices of intercorrelations between age, education, performance test scores, and serum DDE and PBB (males, three age groups, Michigan).

Age, yr	N	r (0.05)	r (0.01)		Correlations						
					Age	Educ.	BD	DS	EF	DDE	PBB
0–29	23	0.39	0.50	Age	+1.00	+0.35	−0.02	−0.16	−0.12	+0.08	+0.27
				Educ.		+1.00	+0.35	+0.12	+0.15	−0.12	−0.02
				BD			+1.00	+0.42	+0.49	+0.02	+0.18
				DS				+1.00	+0.50	−0.35	+0.23
				EF					+1.00	−0.07	+0.13
				DDE						+1.00	+0.32
				PBB							+1.00
30–49	46	0.29	0.37	Age	+1.00	−0.41	−0.20	−0.43	−0.22	+0.21	−0.20
				Educ.		+1.00	+0.34	+0.54	+0.31	−0.20	−0.02
				BD			+1.00	+0.42	+0.60	+0.02	−0.06
				DS				+1.00	+0.30	−0.05	−0.01
				EF					+1.00	+0.08	−0.06
				DDE						+1.00	−0.05
				PBB							+1.00
≥ 50	15	0.48	0.60	Age	+1.00	+0.40	−0.34	−0.45	−0.51	+0.57	+0.69
				Educ.		+1.00	+0.17	+0.10	−0.12	+0.66	+0.64
				BD			+1.00	+0.28	+0.69	−0.30	−0.32
				DS				+1.00	+0.51	−0.41	−0.41
				EF					+1.00	−0.59	−0.47
				DDE						+1.00	+0.94
				PBB							+1.00

Table 2b. Matrices of intercorrelations between age, education, performance test scores, and serum DDE and PBB (females, three age groups, Michigan).

Age, yr	N	r (0.05)	r (0.01)		Correlations						
					Age	Educ.	BD	DS	EF	DDE	PBB
0–29	19	0.43	0.55	Age	+1.00	+0.05	+0.15	−0.14	−0.18	+0.43	+0.19
				Educ.		+1.00	+0.35	+0.48	+0.09	−0.32	−0.43
				BD			+1.00	+1.07	+0.53	+0.16	+0.05
				DS				+1.00	−0.00	−0.20	+0.05
				EF					+1.00	−0.21	+0.14
				DDE						+1.00	+0.34
				PBB							+1.00
30–49	27	0.36	0.47	Age	+1.00	+0.35	−0.14	−0.42	−0.13	+0.47	+0.37
				Educ.		+1.00	+0.01	+0.40	+0.09	+0.07	−0.16
				BD			+1.00	+0.26	+0.38	+0.02	+0.03
				DS				+1.00	+0.32	−0.06	−0.23
				EF					+1.00	−0.15	−0.16
				DDE						+1.00	+0.60
				PBB							+1.00
≥ 50	11	0.55	0.68	Age	+1.00	−0.11	+0.17	−0.34	−0.18	+0.29	−0.36
				Educ.		+1.00	+0.47	+0.42	+0.56	−0.31	+0.17
				BD			+1.00	+0.02	+0.35	−0.14	+0.41
				DS				+1.00	−0.13	−0.04	−0.09
				EF					+1.00	+0.29	−0.15
				DDE						+1.00	−0.21
				PBB							+1.00

Symptoms

Table 3 gives a list of all symptoms recorded on a special questionnaire used in the examination of the Michigan and Wisconsin populations. The questionnaires were administered by physicians; the presence or absence—and, if present, the intensity—of each symptom were recorded for the period preceding the year 1973, and for each consecutive year including 1976.

Biological Indicators of Exposure

PBB in blood serum was analyzed according to the procedure of Liddle, Price, and Bayse *(9)*. Since serum 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (DDE) was readily obtained in these analyses, it has been reported, in addition to PBB. DDE residues in humans are derived from accumulated environmental exposures to DDT and DDE, the latter occurring as a biological dehydrochlori-

Table 3. List of all questions of the Special Questionnaire used to study the comparative prevalence of symptoms in Michigan and Wisconsin for the period 1972–1976.

Eye irritation	Red urine
Nasal irritation	Brown urine
Sore throat	Headaches
Cough	Blurred vision
Wheezing	Dizziness
Tightness in chest	Depression
Skin rash	Tiredness
Acne	Perceptual changes
Sensitivity to sun	Nervousness
Burning sensation on skin	Sleeplessness
Darkening of skin	Sleepiness
Thickening of skin	Muscle weakness
Discoloration or deformation of fingernails	Difficulty in walking
	Paresthesia
Slower or poorer healing of cuts	Loss of balance
Lost more than 10 lb without dieting	Joint pain
Appetite lost	Swelling of joints
Nausea	Pain in lower back and legs
Abdominal pain	Dry skin
Pain related to meals	Thyroid
Crampy abdominal pain	Change of voice
Constipation	Increased perspiration
Diarrhea	

nation product of DDT. Serum obtained from blood in Vacutainers was transferred to glass vials, with Teflon-lined caps, previously washed and rinsed successively with detergent and water, distilled water, acetone and hexane, and frozen until analyzed.

Briefly, a hexane–ether extract (3 × 5 ml) of methanol (2 ml)—treated serum (4 ml) was eluted through a Florisil (1.6 g) column topped with sodium sulfate. The first 13 ml hexane eluate was analyzed by electron-capture gas chromatography (^{63}Ni detector) on a 3 ft × ¼ in. 1% OV101 on 80/100 Supercoport glass column at 240°C. Retention times were approximately 8 min for hexabromobiphenyl, 20 min for heptabromobiphenyl, and 1.5 min for DDE. Recoveries of PBB from serum and standard solutions (hexane) and from serum (spiked with PBB in DMSO) of PBB were 97 ± 12% and 94 ± 11% (mean ± standard deviation) respectively. Analysis for PBB was based on the major peak, 2,4,5-2′,4′,5′-hexabromobiphenyl.

Serum extracts were diluted to less than 500 ng/ml, which was well within the range of linearity of the detector for PBB. The limit of detectability (less than 1000 integrator area units) was ca. 5 pg total injection, and quantitative recovery of PBB from spiked serum samples was successful at 0.2 ppb.

Additional information on the results of PBB determinations in serum for these populations are reported herein (*10, 11*).

Variables Considered in the Analysis of Data

The population examined (Michigan) was grouped according to whether the individuals resided on quarantined or nonquarantined farms or were consumers of products purchased from such farms or workers employed by Michigan Chemical Corporation, manufacturers of the PBB. The description of these groups has been reported (*6*) and a description of behavioral differences in these groups has been discussed (*12*).

Other variables considered in the analysis of neurobehavioral data were: sex, race, age, education, results of behavioral test described above, PBB and DDE in serum, and symptoms before 1973 and in the period 1973–1976 as described above.

Statistical Analysis of Data

Correlation coefficients, computed among all variables, are presented as intercorrelation matrices for both Michigan and Wisconsin residents (Tables 1 and 2). Correlation coefficients were obtained for an equal number of cases within the Michigan group (173) and the Wisconsin group (93). *t*-tests for independent samples were computed to assess male vs. female and male vs. male and female vs. female differences (Tables 4 and 5).

Results

Performance Tests

Figure 1 depicts the mean of scores for three performance tests (BD, DS, and EF) in males (black bars) and females (white bars) in Michigan and Wisconsin for three age groups (0–29, 30–49, and > 50). Serum PBB and DDE levels mean values are also indicated. Statistical differences between males and females at the five and one percent level of significance are indicated by one and two asterisks, respectively. Table 4 contains numerical data corresponding to this illustration.

It was found that females scored better than males in the DS test both in Michigan and Wisconsin in the three age groups. However, for the Michigan subgroup of age 50 and over, males scored worse than females in two additional tests (BD and EF). Serum PBB level differences between males and females are highly significant in all three age groups in Michigan (Table 5).

Table 6 shows the correlation between age, serum PBB, DDE levels, and performance test scores for males and females from Michigan. Analysis of

Table 4. Results of performance tests (BD, DS, and EF) and mean PBB and DDE levels for males and females from Michigan and Wisconsin in three age groups.[a]

		Wisconsin						Michigan					
		Males[a]		Females[b]				Males[c]		Females[d]			
Age, yr		Mean	SD	Mean	SD	*t*	*p*	Mean	SD	Mean	SD	*t*	*p*
0–29	Age	19.1	3.01	2.05	4.83	−0.85	NS	23.3	3.26	22.1	3.81	+.99	NS
	Educ.	2.87	0.93	3.3	0.85	−0.19	NS	3.33	0.66	3.26	0.93	+.25	NS
	BD	29.7	7.5	28.8	5.84	+0.28	NS	30.3	9.7	29.3	7.50	+.34	NS
	DS	54.4	14.9	61.6	7.91	−1.19	NS	50.6	10.4	58.9	6.89	−2.83	<0.005
	EF	34.2	4.3	33.6	4.35	−0.30	NS	34.1	4.4	34.3	4.58	−1.29	NS
	DDE							11.6	8.4	7.67	4.0	+1.81	<0.05
	PBB							60.2	195.6	2.5	2.54	+1.28	NS
30–49	Age	41.9	5.34	38.9	5.16	+1.60	≈0.05	38.91	5.78	38.4	5.74	−0.35	NS
	Educ.	2.94	1.26	3.2	0.61	−0.74	NS	3.01	0.99	2.94	0.81	+0.31	NS
	BD	27.9	9.2	26.9	9.22	+0.30	NS	28.8	8.6	24.8	9.68	+1.83	<0.05
	DS	45.3	10.9	57.0	11.26	−2.95	<0.005	42.2	14.2	56.6	12.46	−4.37	<0.005
	EF	33.6	3.7	32.8	4.55	+0.53	NS	31.4	5.2	33.9	3.08	−2.27	<0.025
	DDE							17.7	10.8	13.8	8.04	+1.62	<0.05
	PBB							37.8	195.0	3.26	6.78	+0.92	NS
≥ 50	Age	58.1	6.10	54.7	10.5	+1.15	NS	57.4	6.27	54.0	2.65	+1.63	<0.05
	Educ.	2.38	.83	2.97	1.06	−1.70	<0.05	2.86	0.91	3.13	0.78	−0.79	NS
	BD	20.9	10.3	23.9	9.19	−0.78	NS	19.7	6.56	26.9	7.48	−2.61	<0.005
	DS	34.0	10.5	50.0	11.02	−3.93	<0.005	30.5	7.81	48.18	10.51	−4.93	<0.005
	EF	27.7	7.7	31.7	4.34	−1.52	≈0.05	24.9	8.15	31.5	5.13	−2.35	<0.025
	DDE							26.6	28.1	17.9	5.11	+1.00	NS
	PBB							27.4	456.7	2.93	1.99	+7.31	> 0.001

[a] For 0–29 yr, $N = 16$; 30–49, $N = 14$; ≥ 50, $N = 22$.
[b] For 0–29 yr, $N = 7$; 30–49, $N = 18$; ≥ 50, $N = 16$.
[c] For 0–29 yr, $N = 16$; 30–49, $N = 46$; ≥ 50, $N = 15$.
[d] For 0–29 yr, $N = 19$; 30–49, $N = 27$; ≥ 50, $N = 11$.

Table 5. Michigan vs. Wisconsin, performance tests and PBB and DDE levels, *t*-tests, male vs. male and female vs. female for three age groups.

		Males						Females					
		Wisconsin[a]		Michigan[b]				Wisconsin[c]		Michigan[d]			
Age, yr		Mean	SD	Mean	SD	*t*	*p*	Mean	SD	Mean	SD	*t*	*p*
0–29	Age	19.1	3.01	23.3	3.26	−4.08	<0.01	20.5	4.83	22.1	3.81	−0.88	NS
	Educ.	2.87	0.93	3.33	0.66	−1.81	NS	3.34	0.85	3.26	0.93	+0.20	NS
	BD	29.7	7.5	30.3	9.7	−0.21	NS	28.8	5.84	29.3	7.50	−1.58	NS
	DS	54.4	14.9	50.6	10.4	−0.94	NS	61.6	7.91	58.9	6.89	+0.85	NS
	EF	34.2	4.3	34.1	4.4	−0.07	NS	33.6	4.35	34.3	4.68	−0.34	NS
	DDE			11.6	8.4					7.67	4.0		
	PBB			60.2	195.6					2.52	2.54		
30–49	Age	41.9	5.34	38.91	5.78	+1.72	<.05	38.9	5.16	39.4	5.74	−0.30	NS
	Educ.	2.94	1.26	3.01	0.99	−0.22	NS	3.19	0.61	2.94	0.81	+1.14	NS
	BD	27.9	9.2	28.8	8.6	−0.33	NS	26.9	9.22	24.8	9.68	+0.72	NS
	DS	45.3	10.9	42.2	14.2	+0.75	NS	57.0	11.26	56.6	12.46	+0.11	NS
	EF	33.6	3.7	31.4	5.2	+1.46	NS	32.8	4.54	33.9	3.08	−0.97	NS
	DDE			17.7	10.8					13.8	8.04		
	PBB			37.8	195.0					3.26	6.78		
≥ 50	Age	58.1	6.10	57.4	6.27	+0.34	NS	54.7	10.5	54.0	2.55	+0.22	NS
	Educ.	2.38	.83	2.86	0.91	−1.66	<0.05	2.97	1.06	3.13	0.78	−0.43	NS
	BD	20.9	10.3	19.7	6.56	+0.40	NS	23.9	9.19	26.9	7.48	−0.90	NS
	DS	34.0	10.5	30.5	7.81	+1.09	NS	50.0	11.02	48.18	10.51	+0.43	NS
	EF	27.7	7.7	24.9	8.15	+1.06	NS	31.7	4.34	31.5	5.13	+0.11	NS
	DDE			26.6	28.1					17.9	5.11		
	PBB			127.4	456.7					2.93	1.99		

[a] For age 0–29 yr, $N = 16$; 30–49, $N = 14$; ≥ 50, $N = 22$.
[b] For age 0–29 yr, $N = 23$; 30–49, $N = 46$; ≥ 50, $N = 15$.
[c] For age 0–29 yr, $N = 7$; 30–49, $N = 18$; ≥ 50, $N = 16$.
[d] For age 0–29 yr, $N = 19$; 30–49, $N = 27$; ≥ 50, $N = 11$.

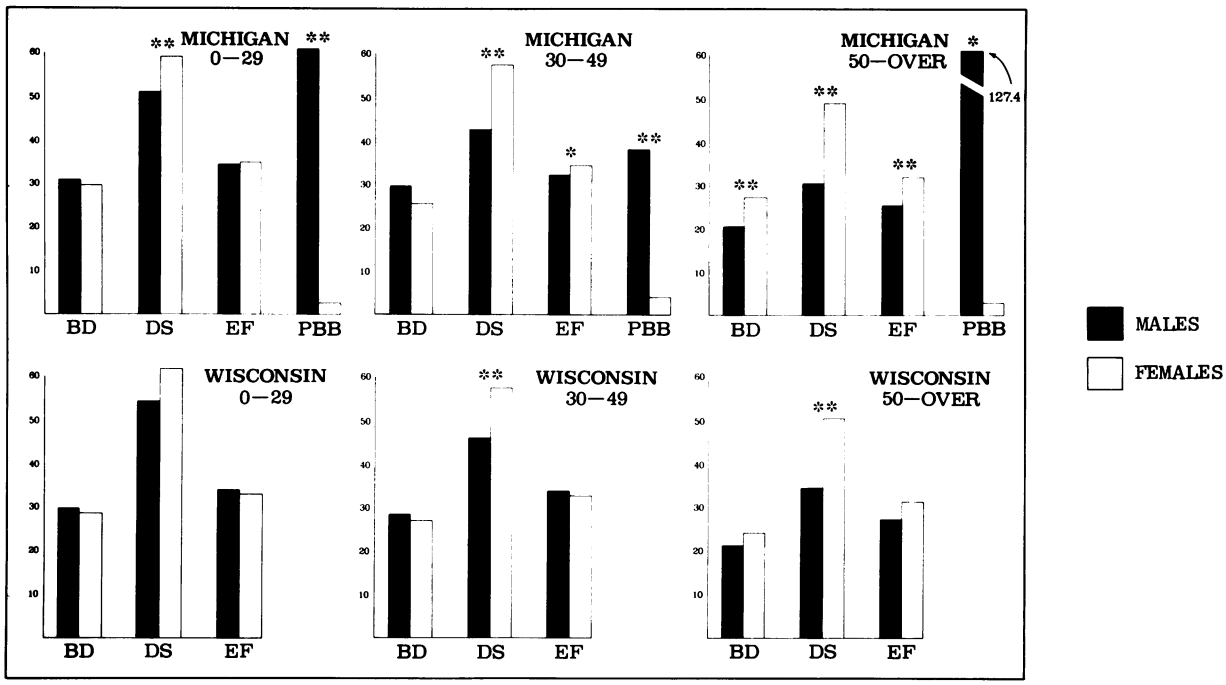

FIGURE 1. Results of three performance tests—Block Design (BD), Digit—Symbol (DS) and Embedded Figures (EF)—in males and females in Michigan and Wisconsin for three age groups as shown. Asterisks indicate differences which are significant at the 1% and 5% levels respectively. Note that while in Wisconsin females scored better than males in one test (DS), in Michigan and particularly in the 50 and over group, males scored worse than females in all tests. Serum PBB levels were significantly higher in males than females in all age groups.

Table 6. Wisconsin vs. Michigan: matrices of intercorrelations between age, education, performance test scores and serum DDE and PBB levels (males and females, all ages).

Sex	Residence	*N*	*r* (0.05)	*r* (0.01)		Age	Educ.	BD	DS	EF	DDE	PBB
M	Wisconsin	52	0.27	0.35	Age	+1.00	−0.26	−0.41	−0.64	−0.51		
					Educ.		+1.00	+0.23	+0.53	+0.22		
					BD			+1.00	+0.42	+0.56		
					DS				+1.00	+0.51		
					EF					+1.00		
					DDE						+1.00	
					PBB							+1.00
F	Wisconsin	41	0.30	0.39	Age	+1.00	−0.17	−0.40	−0.53	−0.29		
					Educ.		+1.00	+0.12	+0.20	+0.23		
					BD			+1.00	+0.24	+0.45		
					DS				+1.00	+0.29		
					EF					+1.00		
					DDE						+1.00	
					PBB							+1.00
M	Michigan	84	0.20	0.26	Age	+1.00	−0.22	−0.39	−0.57	−0.53	+0.42	+0.15
					Educ.		+1.00	+0.33	+0.44	+0.22	+0.05	+0.19
					BD			+1.00	+0.49	+0.63	−0.17	−0.09
					DS				+1.00	+0.49	−0.27	−0.07
					EF					+1.00	−0.36	−0.27
					DDE						+1.00	+0.55
					PBB							+1.00
F	Michigan	57	0.27	0.35	Age	+1.00	−0.13	−0.14	−0.31	−0.24	+1.61	+0.15
					Educ.		+1.00	+0.22	+0.38	+0.81	−0.12	−0.09
					BD			+1.00	+0.18	+0.38	−0.08	+0.04
					DS				+1.00	+0.18	−0.21	−0.16
					EF					+1.00	−0.16	−0.07
					DDE						+1.00	+0.46
					PBB							+1.00

similar data for the Wisconsin population is in progress. Group size and critical values for the evaluation of the significance of the correlation coefficients are shown at the bottom of each column.

With one exception (the correlation coefficient of the BD test with serum PBB in females), correlation coefficients between performance test scores and serum PBB levels were negative, suggesting that a higher serum PBB level was associated with a lower performance test score. The only statistically significant correlation was that for EF test scores among males. Two test scores (DS and EF) were significantly and negatively correlated with serum DDE levels. Tables 1 and 2 contain the complete correlation matrices from which these data were derived. Table 7 shows the correlation coefficients between serum PBB and DDE levels and performance test scores for Michigan males in three age groups. Group size and critical values for the evaluation of the significance of the correlation coefficients are shown at the bottom of each column.

In males, the correlation coefficient between serum PBB levels and age showed a negative trend in the three age groups (+ 0.27, − 0.20, and − 0.69); no similar trend was observed in females (+ 0.19, + 0.37, and − 0.36). As previously shown, the mean serum PBB levels for males were significantly higher than for females. In males, the correlation coefficient between serum DDE levels and age was increasingly positive (+ 0.08, + 0.21, and + 0.57) in the three consecutive age groups (statistically significant for the older group). In females, serum DDE level was significantly correlated with age in the two younger groups.

The correlation coefficients between BD, DS and EF test and serum PBB levels in males were progressively more negative as a function of age group but were not statistically significant. For the BD test, the values were + 0.18, − 0.16, and − 0.32; for the DS test, + 0.23, − 0.007, and − 0.41; and for the EF test, + 0.13, − 0.16, and − 0.57, respectively. No similar trend was observed in the magnitude of the correlation coefficients between test scores and serum PBB levels in females as a function of age group. A similar analysis for trends in the correlation coefficients between serum DDE levels and test scores revealed that these coefficients were negative and in one case (EF) significant in males of the oldest age group. No similar pattern was detected in females.

The behavioral findings analyzed by means of correlational techniques were convergent with those obtained from assessment of population differences (Fig. 1): severity of central nervous system dysfunction, as evaluated by means of performance tests increased with age in the male group.

Neurological Symptoms

Figure 2 shows the percentage of cases exhibiting neurological symptoms in Michigan (white bars) and Wisconsin (black bars) in a subsample of the population also examined by means of performance tests. The total numbers of people so examined in Michigan and Wisconsin were 170 and 93, respectively. While the number of reported symptoms during the period 1972-1976 varied little in Wisconsin, in Michigan there was a marked and significant increase in these symptoms during the same period. In this illustration the symptoms are ordered according to the reported frequency of occurrence during the last year (1976). It is interesting to note that certain notable symptoms such as tiredness, headaches, sleepiness, nervousness, dizziness, blurred vision, muscle weakness and loss of balance were reported less by Michigan residents for 1972 than by the Wisconsin group. Numerical data corresponding to this illustration are to be found in Tables 8-10.

Table 7. Michigan: Correlation between DDE and PBB and performance test scores in males and females in three age groups.

	Age 0–29				Age 30–49				Age ≥ 50			
	Males		Females		Males		Females		Males		Females	
	DDE	PBB	DDE	PBB	DDE	PBB	DDE	PBB	DDE	PBB	DDE	PBB
Age	+0.08	+0.27	+0.43	+0.19	+0.21	−0.20	+0.47	+0.37	+0.57	−0.69	−0.29	−0.36
BD	+0.02	+0.18	+0.16	+0.05	+0.02	−0.06	+0.02	+0.03	−0.30	−0.32	−0.14	+0.41
DS	−0.35	+0.23	−0.20	+0.05	−0.05	−0.007	−0.06	−0.23	−0.41	−0.41	−0.04	−0.09
EF	−0.07	+0.13	−0.21	−0.14	+0.08	−0.06	−0.15	−0.16	−0.59	−0.57	+0.29	−0.15
DDE	—	+0.32	—	+0.34	—	−0.05	—	+0.60	—	+0.94	—	−0.21
N	23		19		46		27		15		11	
r (0.05)	0.39		0.43		0.29		0.36		0.48		0.55	
r (0.01)	0.50		0.55		0.37		0.47		0.60		0.68	

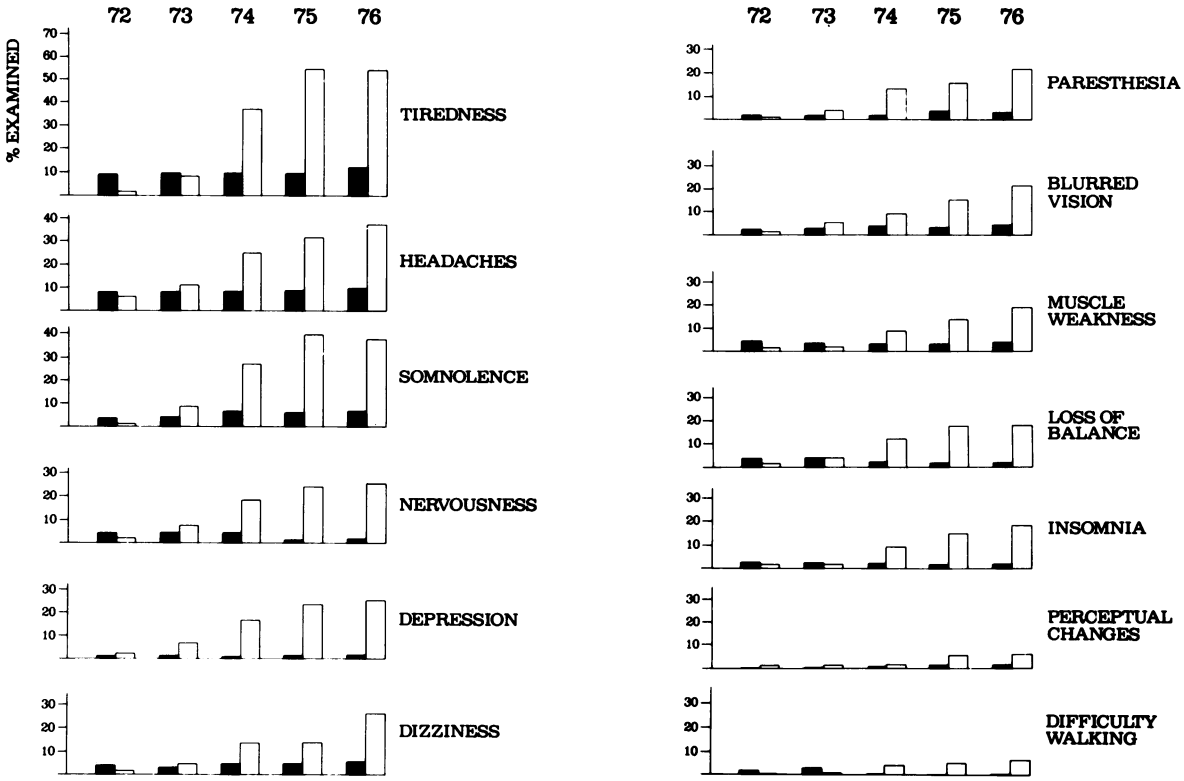

FIGURE 2. Proportion of cases exhibiting neurological symptoms in (□) Michigan and (■) Wisconsin in subsamples of the study populations examined by means of performance tests. The total number of examined individuals in Michigan and Wisconsin was 170 and 93, respectively. While in the Wisconsin group the number of reported symptoms during the period 1972-1976 was stable, there was marked increase in these symptoms in the Michigan group in the same period.

Table 8. Time course of prevalence of neurological symptoms among Wisconsin residents (N = 92) as obtained by history.

	1972		1973		1974		1975		1976	
	No.	%	No.	%	No.	%	No.	%	No.	%
Tiredness	9	9.7	9	1.7	9	9.7	9	9.7	11	11.9
Headaches	7	7.6	7	7.6	7	7.6	8	8.7	8	8.7
Sleepiness	3	3.3	4	4.3	5	5.4	5	5.4	5	5.4
Nervousness	3	3.3	3	3.3	3	3.3	1	1.1	1	1.1
Depression	1	1.1	1	1.1	1	1.1	1	1.1	2	2.2
Dizziness	3	3.3	3	3.3	4	4.3	4	4.3	5	5.4
Paresthesia	2	2.2	2	2.2	2	2.2	4	4.3	3	3.3
Blurred vision	3	3.3	3	3.3	4	4.3	3	3.3	4	4.3
Muscle weakness	5	5.4	4	4.3	3	3.3	3	3.3	3	3.3
Loss of balance	4	4.3	4	4.3	2	2.2	1	1.1	1	1.1
Sleeplessness	2	2.2	2	2.2	2	2.2	1	1.1	2	2.2
Perceptual change	0	0.0	0	0.0	1	1.1	1	1.1	2	2.2
Difficulty walking	2	2.2	3	3.3	1	1.1	0	0.0	0	0.0

Figure 3 illustrates the method used to calculate the approximate time of onset of each symptom. The proportions of persons with and without symptoms in the Michigan and Wisconsin populations were compared for each year (1972-1976) by using the chi-square test. The chi-square value was plotted against time and the statistical significance at the 0.05 and 0.01 level was indicated by the two parallel lines. The points of intersect of these lines with the curve resulting from successive chi-square values indicate the time at which a symptom became significantly more prevalent in the Michigan

Table 9. Time course of prevalence of neurological symptoms among Michigan residents (N = 166) as obtained by history.

	1972		1973		1974		1975		1976	
	No.	%	No.	%	No.	%	No.	%	No.	%
Tiredness	2	1.2	16	9.6	62	37	88	53	90	54
Headaches	10	6.0	17	10.2	41	25	53	32	64	39
Sleepiness	1	0.6	13	7.8	45	27	63	38	62	37
Nervousness	3	1.8	12	7.2	30	18	40	24	44	27
Depression	3	1.8	11	6.6	29	17	38	23	42	25
Dizziness	1	0.6	9	4.5	22	13	22	13	43	26
Paresthesia	3	1.8	7	4.2	22	13	26	16	36	22
Blurred vision	2	1.2	9	5.4	16	10	27	16	36	22
Muscle weakness	1	0.6	4	2.4	15	9	24	14	31	19
Loss of balance	2	1.2	7	4.2	20	12	29	17	30	18
Sleeplessness	1	0.6	4	2.4	15	9.0	25	15	30	18
Perceptual change	1	0.6	2	1.2	5	3.0	10	6.0	10	6.0
Difficulty walking	0	0.0	2	1.2	6	3.6	8	4.8	10	6.0

Table 10. Comparative percentages of prevalence of neurological symptoms among Wisconsin (W) and Michigan (M) residents between 1972 and 1976 as obtained by history.

	1972		1973		1974		1975		1976	
	W, %	M, %	W, %	M, %	W, %	M, %	W, %	M, %	W, %	M, %
Tiredness	9.7	1.2	9.7	9.6	9.7	37	9.7	53	11.9	54
Headaches	7.6	6.0	7.6	10.2	7.6	25	8.7	32	8.7	39
Sleepiness	3.3	0.6	4.3	7.8	5.4	27	5.4	38	5.4	37
Nervousness	3.3	1.8	3.3	7.2	3.3	18	1.1	24	1.1	27
Depression	1.1	1.8	1.1	6.6	1.1	17	1.1	23	2.2	25
Dizziness	3.3	0.6	3.3	4.5	4.3	13	4.3	13	5.4	26
Paresthesia	2.2	1.8	2.2	4.2	2.2	13	4.3	16	3.3	22
Blurred vision	3.3	1.2	3.3	5.4	4.3	10	3.3	16	4.3	22
Muscle weakness	5.4	0.6	4.3	2.4	3.3	9	3.3	14	3.3	19
Loss of balance	4.3	1.2	4.3	4.2	2.2	12	1.1	17	1.1	18
Sleeplessness	2.2	0.6	2.2	2.4	2.2	9	1.1	15	2.2	18
Perceptual change	0.0	0.6	0.0	1.2	1.1	3	1.1	6	2.2	6
Difficulty walking	2.2	0.0	3.3	1.2	1.1	4	0.0	5	0.0	6

population. By this method it was possible to determine the approximate time of onset of the various symptoms reported by the examined persons at the five and one percent level of significance.

The curves shown in Figure 4 represent the growth of the magnitude of the chi-square for individual symptoms as explained above. All symptoms for which a significant difference between the Michigan and Wisconsin examined population was found in 1976 are represented in decreasing order of magnitude of the chi-square value. It was found that neurological complaints represented the leading symptoms. All seemed to be reaching a plateau in 1976 but none decreased significantly over the years.

Figure 5 illustrates the time course of each symptom as determined by the method described above. A temporal sequence was reconstructed according to their relative time of onset (the number in the scale indicates months as relative units). No attempt was made to determine the precise time of onset of the symptom although the relative time of onset could be ascertained. The dashed and solid lines indicate areas where the criterion for significance of 5 and 1%, respectively, was met. All the symptoms without exception listed in Table 3 were considered for this analysis, and only those showing significant differences when compared to the Wisconsin population are shown.

In the Michigan population, the onset of neurological symptoms preceded all others. The frequency of some of the symptoms, such as nausea and dizziness, increased slowly over the months whereas others, such as depression, nervousness, paresthesia, tiredness, sleepiness and headaches showed a more rapid increase in prevalence. Interestingly, "motor involvement" such as loss of balance, muscle weakness, difficulty in walking, and blurred vision occurred relatively later in time or showed a slower rate of increase. Finally, the

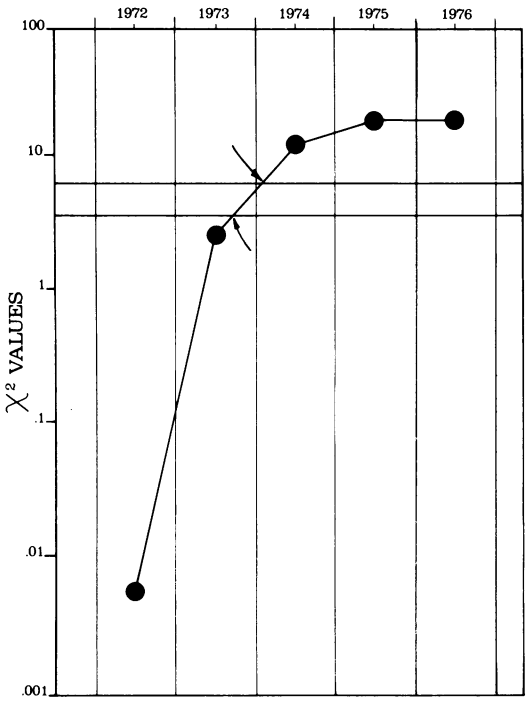

FIGURE 3. Illustration of the method used to calculate the time of onset of symptoms in the Michigan population. Proportion of persons with and without symptoms in the Michigan and Wisconsin population were compared for each year (1972–1976) by using the chi-square test. The value of the chi-square was plotted against time and the statistical significance at the 0.05 and 0.01 levels is indicated by the two parallel horizontal lines. The points of intersect of these lines with the curve resulting from successive chi-square values indicate the time at which a symptom became significantly more prevalent in the Michigan population. By this method is was possible to determine the approximate time of onset of the various symptoms reported by the group at the 5% and 1% levels of significance.

prevalence of symptoms such as increased perspiration, slower healing of cuts, and nail discoloration progressed slowly during 3–7 months. Some of these symptoms, such as increased perspiration, swelling of joints, and difficulty in walking were never reported significantly higher than at the 5% level, compared to the Wisconsin population.

Tables 11 and 12 are summaries of data. Table 12 lists significant symptoms as observed in November of 1976. The left column indicates proportion of cases in Michigan listed in decreasing order of significance. The column on the right is a list of significant symptoms as detected by the chi-square test, also listed in decreasing order of significance.

Table 12 contains the evolution of symptoms as reconstructed from the special questionnaire and the method described above: the higher the position in the list, the earlier its occurrence. It is shown that the order in which symptoms have established themselves varies according to whether a less conservative criterion (5%) or a more conservative criterion (1%) was taken. However, in both cases neurological symptoms were the earliest manifestation of abnormalities in the Michigan population.

Discussion

Preliminary analysis of data reported here indicates that a significant constellation of neurological symptoms occurred among dairy farmers during the period 1972–1976, when patterns for a non-PBB-exposed dairy farm population are taken as a reference. Simple statistical procedures demonstrate that neurological symptoms were also likely to be the earliest manifestations of abnormalities among the Michigan dairy farmers. Performance test results suggest that in older males, higher serum PBB levels were more likely to be associated with dysfunction.

Serum PBB levels are significantly higher in males than in females in all age groups and, in general, performance test scores are lower in males than in females. However, since in some tests, such as the DS test, women scored better than males in the reference population also, interpretation of these results should be made with caution. However, after considering these factors, there is a distinctive group of males (50 and over) which performed significantly less well than the control population.

Analysis of correlations between symptoms and serum PBB levels in males and females of various age groups and between performance tests and neurological symptoms is still in progress. PBB levels in depot fat biopsies are expected to be available and will add another dimension to this analysis. It is possible that fat PBB levels may be even more closely related to neurobehavioral symptoms and behavioral test scores.

As determined at one specific point in time (November 1976), neurological symptoms that are significantly more prevalent in Michigan seemed to have reached a plateau.

We attempted to eliminate descriptions of anecdotal type, although the descriptions—such as numerous reports of marked memory difficulties—illuminate the symptomatology that we describe here only briefly. In this report, our analysis was restricted to those data which could be documented as objectively as possible.

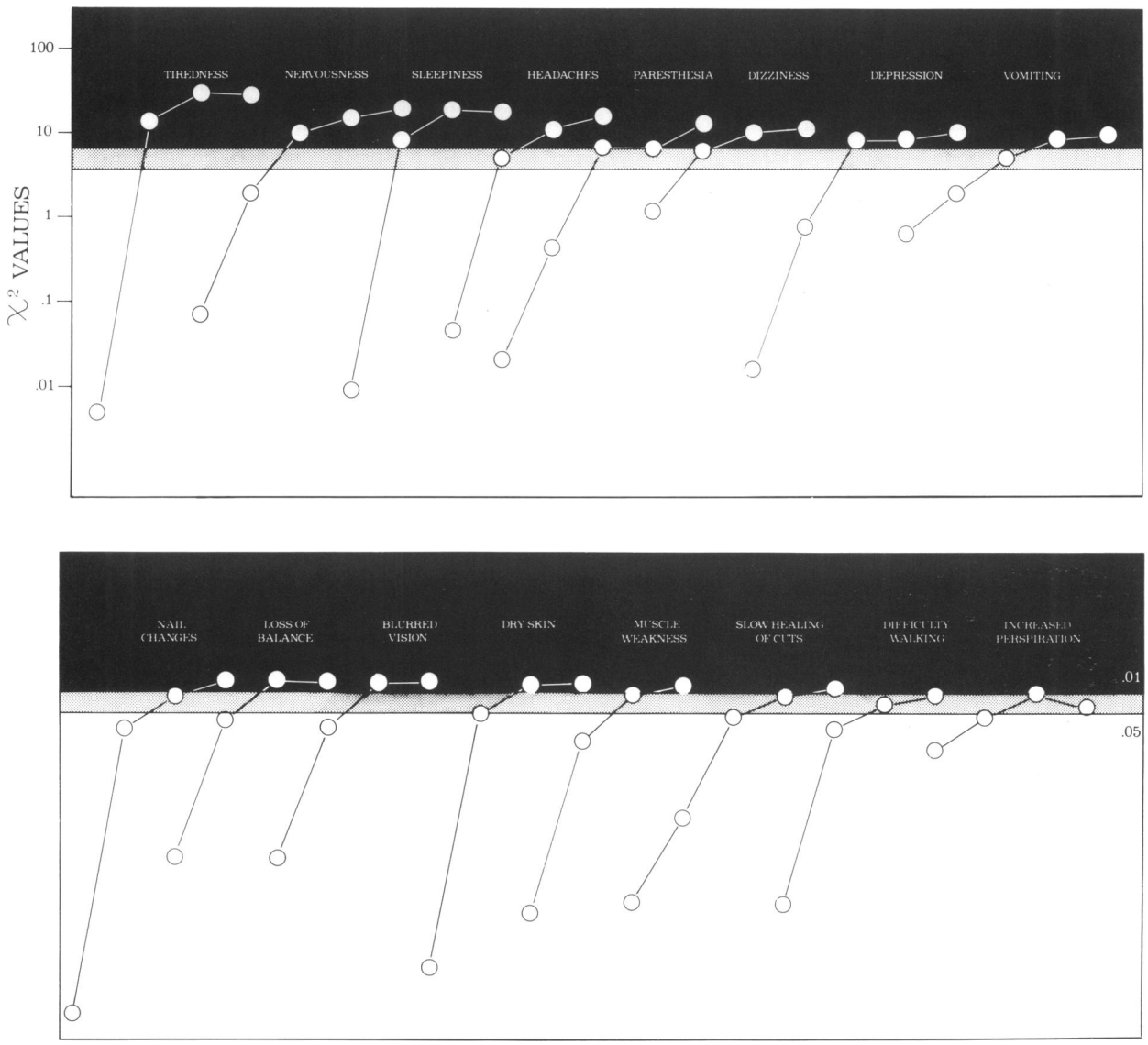

FIGURE 4. Changes in chi-square values over time for individuals symptoms according to the method described in Fig. 3. All symptoms in which there was a significant difference between the Michigan and Wisconsin populations in 1976 are represented. As can be seen, neurological symptoms led. All seemed to reach a plateau but none decreased over the years so far studied.

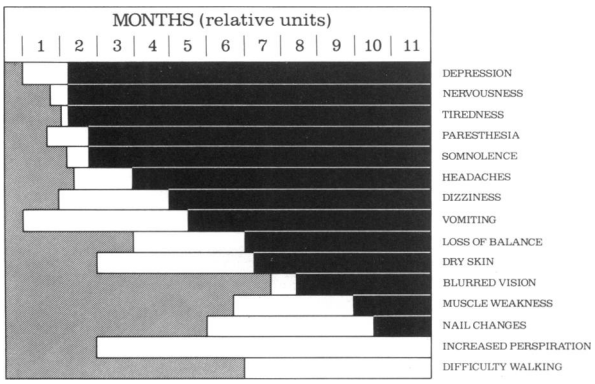

FIGURE 5. Time of onset of all significant symptoms in the Michigan population. The time of onset was determined by means of the method described in Fig. 3. The upper scale indicates months as a relative unit. Although no attempt was made to determine the precise time of onset of the symptoms, their onset could be ascertained with respect to each other. Significance at the 5% and 1% level are indicated by white and dashed bars, respectively, and the arrangement is made according to the 1% level of significance.

Table 11. Symptoms observed among Michigan dairy farmers in 1976.

Proportion of cases (1976, Michigan)	Significance of difference between groups
Tiredness	Tiredness
Headaches	Nervousness
Sleepiness	Sleepiness
Drier skin	Headaches
Nervousness	Paresthesia
Paresthesia	Dizziness
Perspiration	Depression
Dizziness	Vomiting
Depression	Nail discoloration
Blurred vision	Loss of balance
Muscle weakness	Blurred vision
Loss of balance	Drier skin
Healing cuts	Muscle weakness
Vomiting	Healing cuts
Nail discoloration	Difficulty walking
Difficulty walking	Perspiration
Swelling of joints	Swelling of joints

Table 12. Sequence of appearance of symptoms among Michigan dairy farmers.

5% criterion	1% criterion
Depression	Depression
(Nausea) vomiting	Nervousness
Nervousness	Tiredness
Paresthesia	Paresthesia
Tiredness	Sleepiness
Dizziness	Perspiration
Sleepiness	Headaches
Headaches	Dizziness
Perspiration	Vomiting
Drier skin	Loss of balance
Loss of balance	Difficulty walking
Healing cuts	Drier skin
Nail discoloration	Blurred vision
Muscle weakness	Muscle weakness
Difficulty walking	Nail discoloration
Blurred vision	Healing cuts

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